

SUPPLEMENTAL FIGURES

Spatial N-glycomics of the Human Aortic Valve in Development and Pediatric Endstage Congenital Aortic Valve Stenosis

Peggi M. Angel¹, Richard Drake¹, Yeonhee Park², Cassandra L. Clift¹, Connor West¹, Savanna Berkhiser¹, Anand Mehta¹, David Bichell³, Yan Ru Su⁴

1, Department of Cell and Molecular Pharmacology; MUSC Proteomics Group; Bruker Clinical Glycomics Center of Excellence, Medical University of South Carolina, Charleston, SC

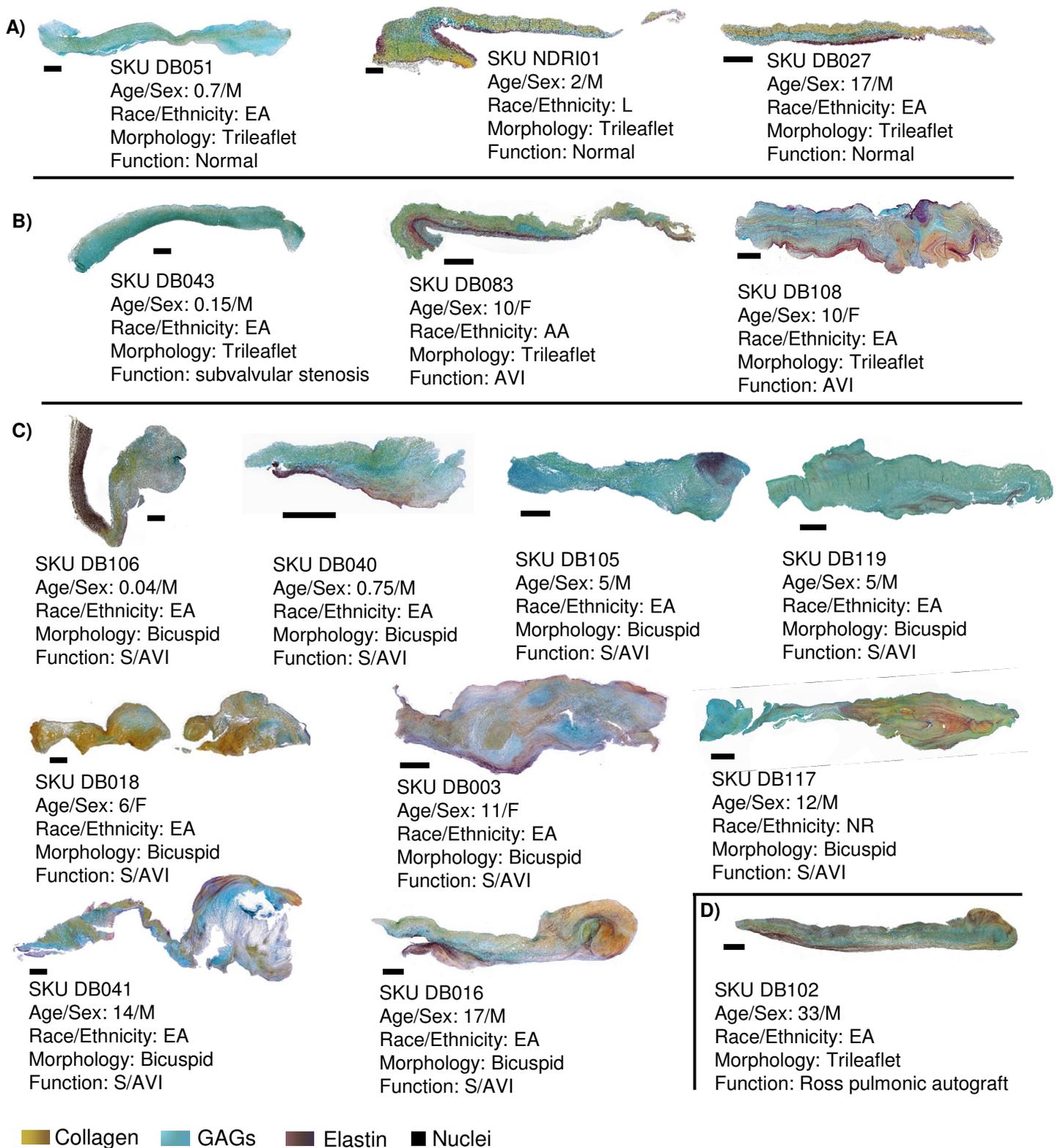
2, University of Wisconsin-Madison, Department of Biostatistics and Medical Informatics, Madison WI

3, Division of Pediatric Cardiac Surgery, Vanderbilt University Medical Center, Nashville, Tennessee

4, Division of Cardiovascular Medicine, Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee

Corresponding Author: Peggi M. Angel, 173 Ashley Ave, BSB358, Charleston, SC 29425
Email: angelp@musc.edu Phone: [\(843\) 792-8410](tel:(843)792-8410) Fax: [\(843\) 792-0481](tel:(843)792-0481)

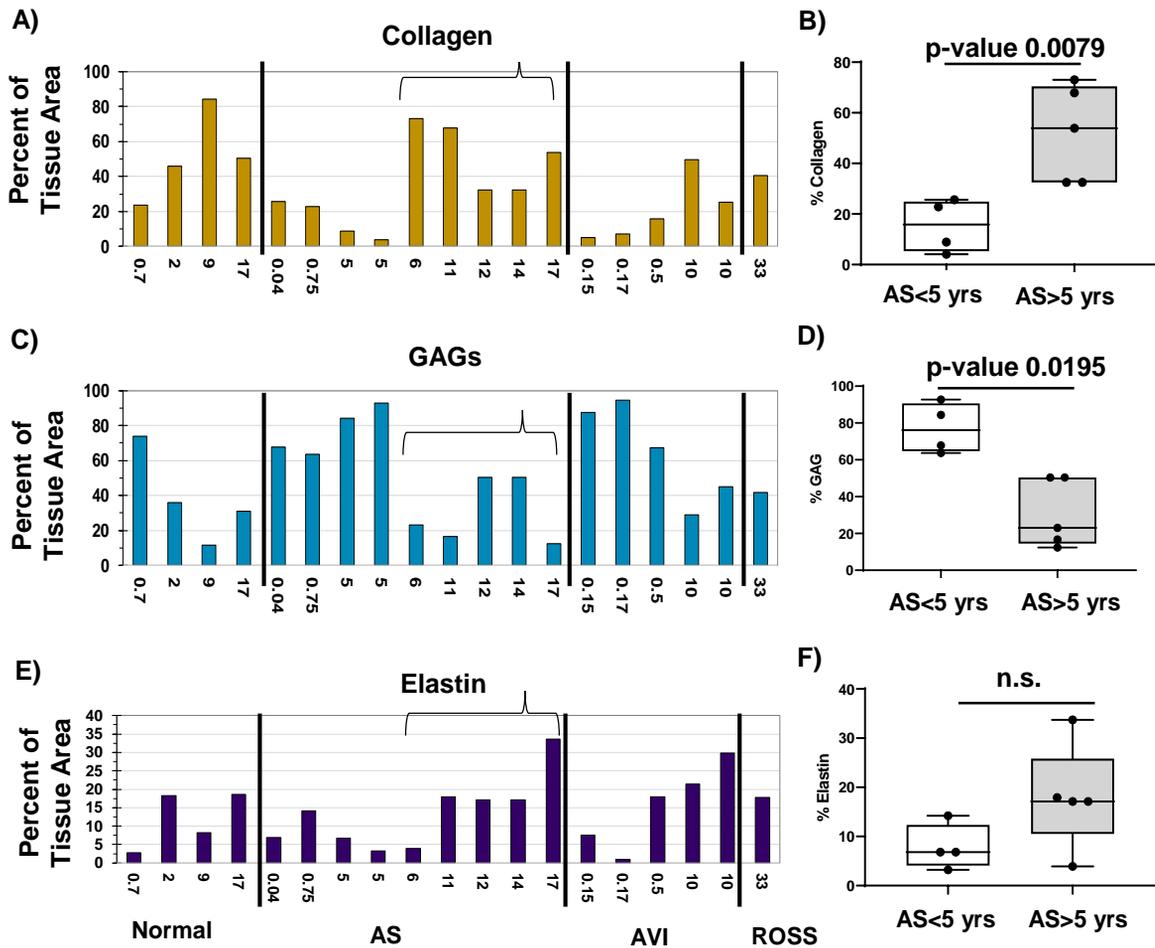
Supplemental Figure 1



Supplemental Figure 1. Extracellular matrix organization in aortic valves used for the spatial N-glycomics study.

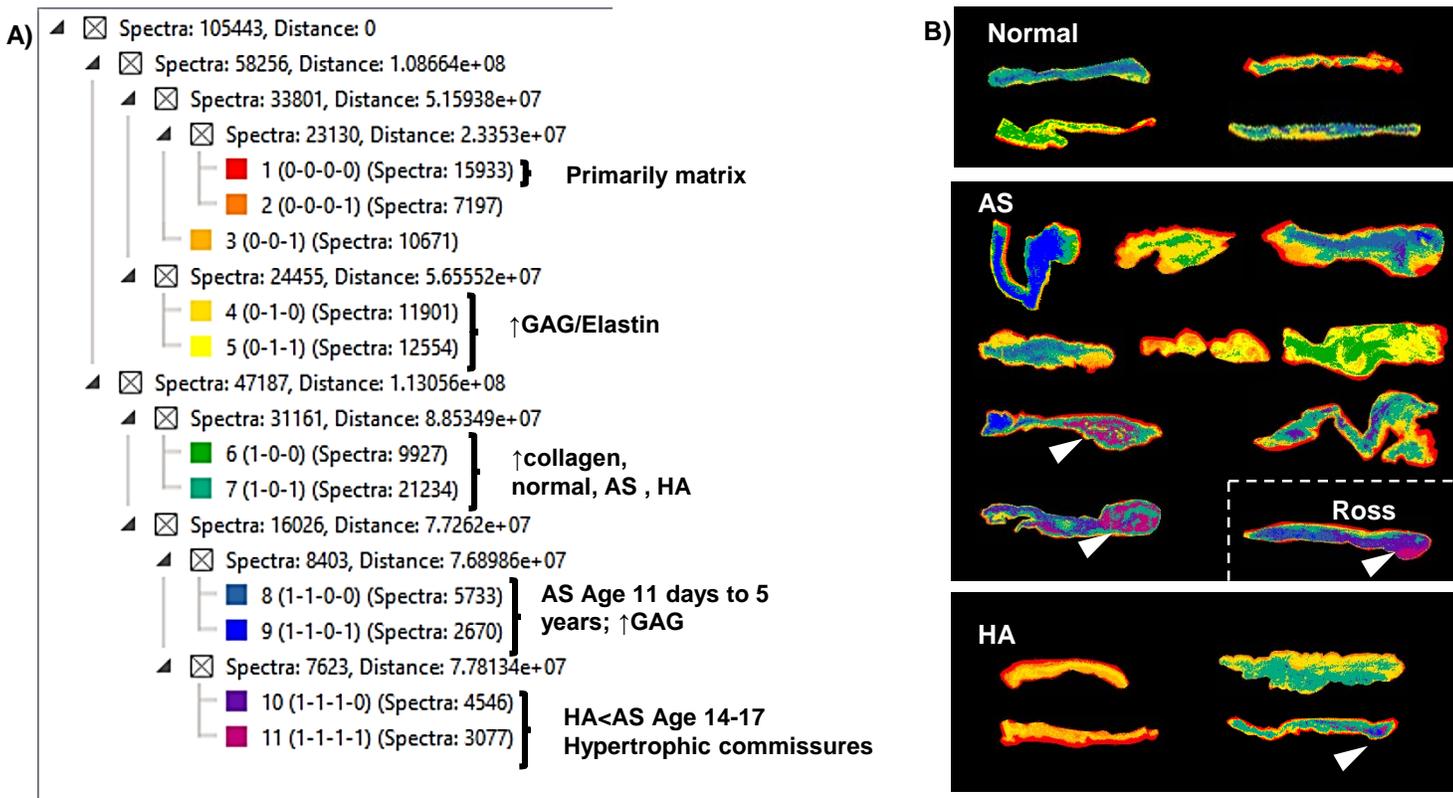
A) Examples of aortic valves with trileaflet structure and normal function. B) Examples of aortic valves with trileaflet structure operating under altered hemodynamics. C) Examples of aortic valves with pediatric endstage aortic valve stenosis. All surveyed valves were bicuspid with aortic valve insufficiency. D) A Ross pulmonic autograft valve was included in the study for comparison to the failed AV. Abbreviations: AVI- aortic valve insufficiency; S- stenotic AVI- aortic valve insufficiency; EA- European American; L- latino; AA- African-American; Sex M- male; Sex F- female. Bar = 300 μ m. SKUs with abbreviation DB are from the Vanderbilt University and Medical Center Heart Registry and Biorepository. SKUs with abbreviation NDRI are from the National Disease Research Interchange.

Supplemental Figure 2

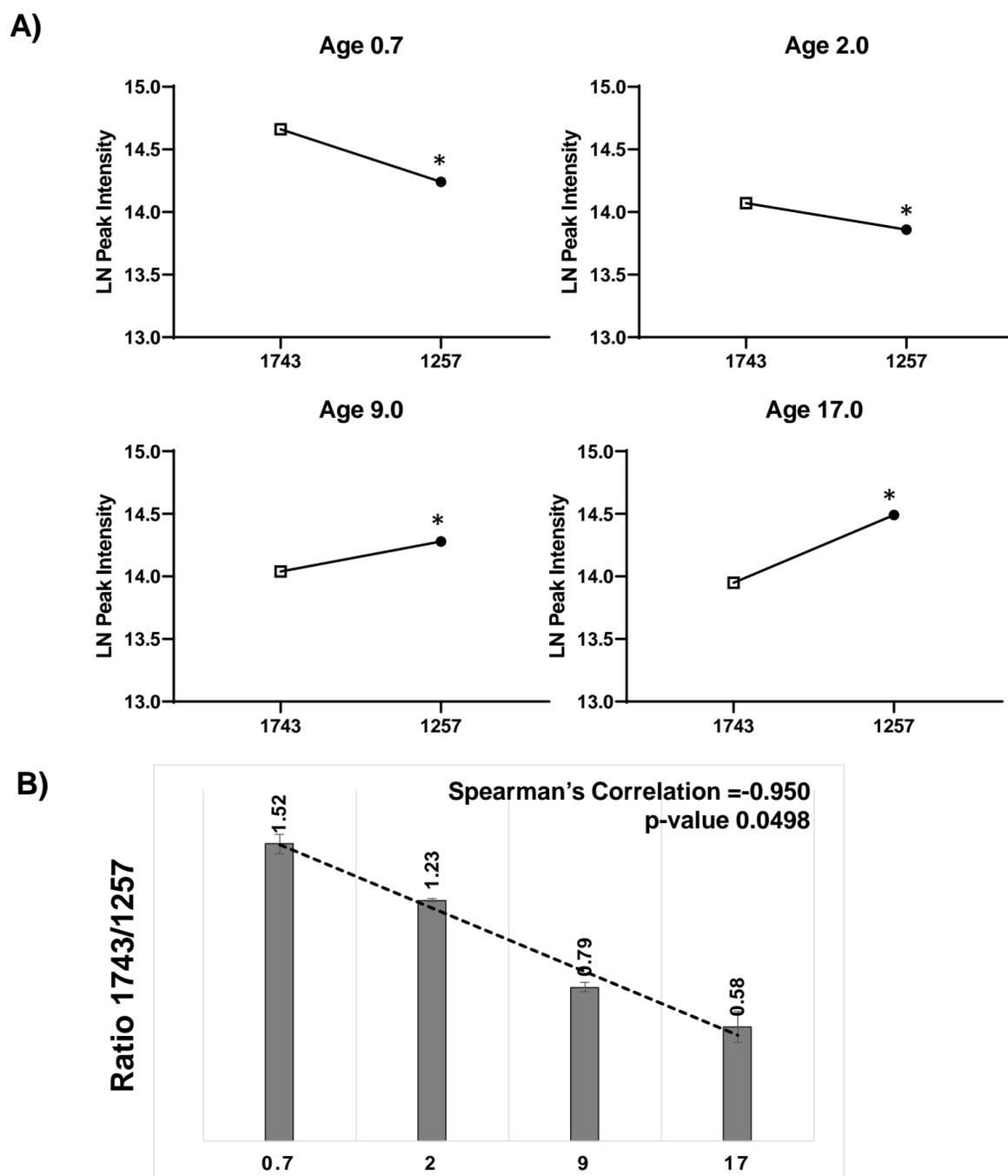


Supplemental Figure 2. Patient-specific ECM characterization based on Movat's pentachrome staining. Movat's pentachrome stains collagens yellow-yellow/orange, glycosaminoglycans blue, elastin purple to black and nuclei black. Smooth muscle tissue stains red, which was not present in valve tissue. A) Percentage of tissue area staining for collagen by patient. B) The AS group > 5 years showed a significantly higher level of collagen, Mann Whitney U p-value 0.0079. C) Glycosaminoglycan (GAG) staining per patient. D) Using an alpha 0.05 as a cutoff for significance, there was a significant difference in GAG by AS age group (Mann Whitney U p-value 0.0195). E) Patient specific elastin staining. F) There was no significant difference in GAG by AS age group (Mann Whitney U p-value 0.127). Statistical testing was done in GraphPad Prism v8.4.3.

Supplemental Figure 3

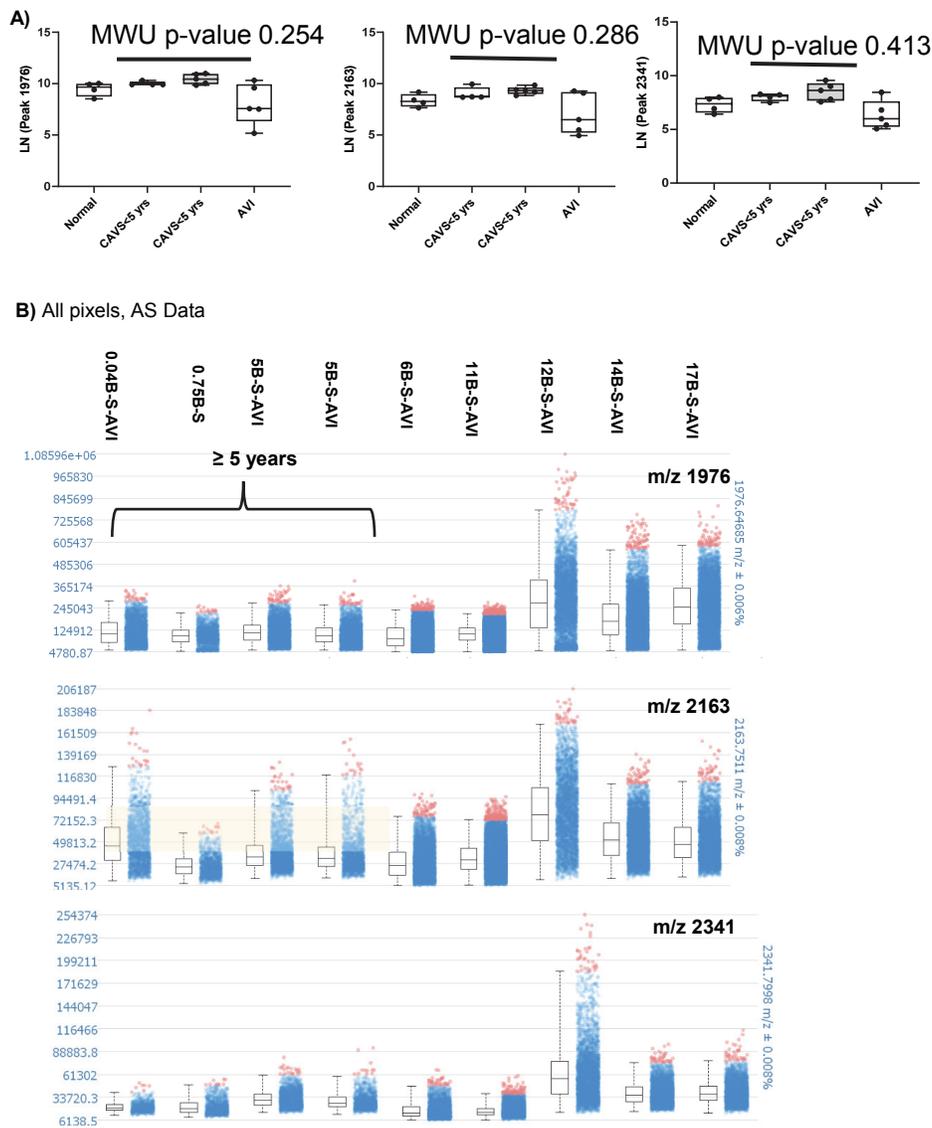


Supplemental Figure 3. Spatial Hierarchical clustering of the N-glycomes shows that N-glycomes are spatially organized within the valvular structure. A) Extended hierarchical clustering of spectra. The data pixels collected across each tissue are grouped by location and intensity within each tissue. Each group is colorized to demonstrate distinct localization of N-glycomes. B) Localization of each group across all human aortic valve tissues. The distinct patterns are similar to the extracellular matrix staining by Movat's pentachrome stain.



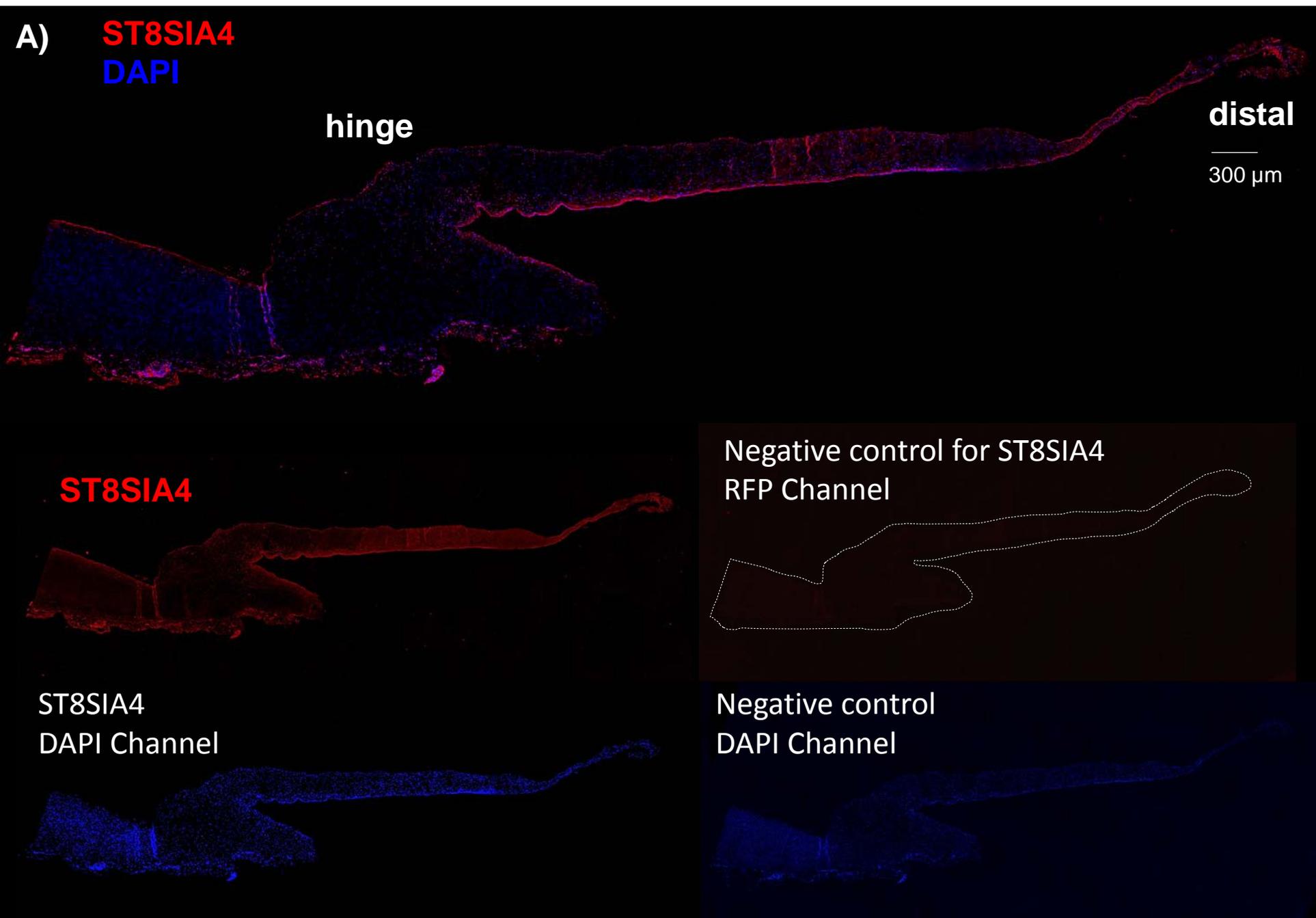
Supplemental Figure 4. Ratio comparison of Man8 (m/z 1743), an N-glycan structure occurring during glycoprotein folding in the E.R., and Man5, a trimmed mannose structure that occurs after export of the glycoprotein from the E.R. to the cis-golgi. A) Trendline comparison of Man8/Man5 ratios at each age. Data was compared using the natural log normalized mean and relative standard deviation of N-glycan peak intensity across each valve. The Mann-Whitney U test was used for statistical comparison between Man8 and Man5. * signifies p-value <0.0001 for comparison. B) Spearman's correlation shows a significant correlation between the ratio of Man8/Man5 and age of the valve. Man8 decreases as the valve ages suggesting increases in processes of mannose trimming. Mannose trimming is a prerequisite to further extension of the N-glycan.

Supplemental Figure 5



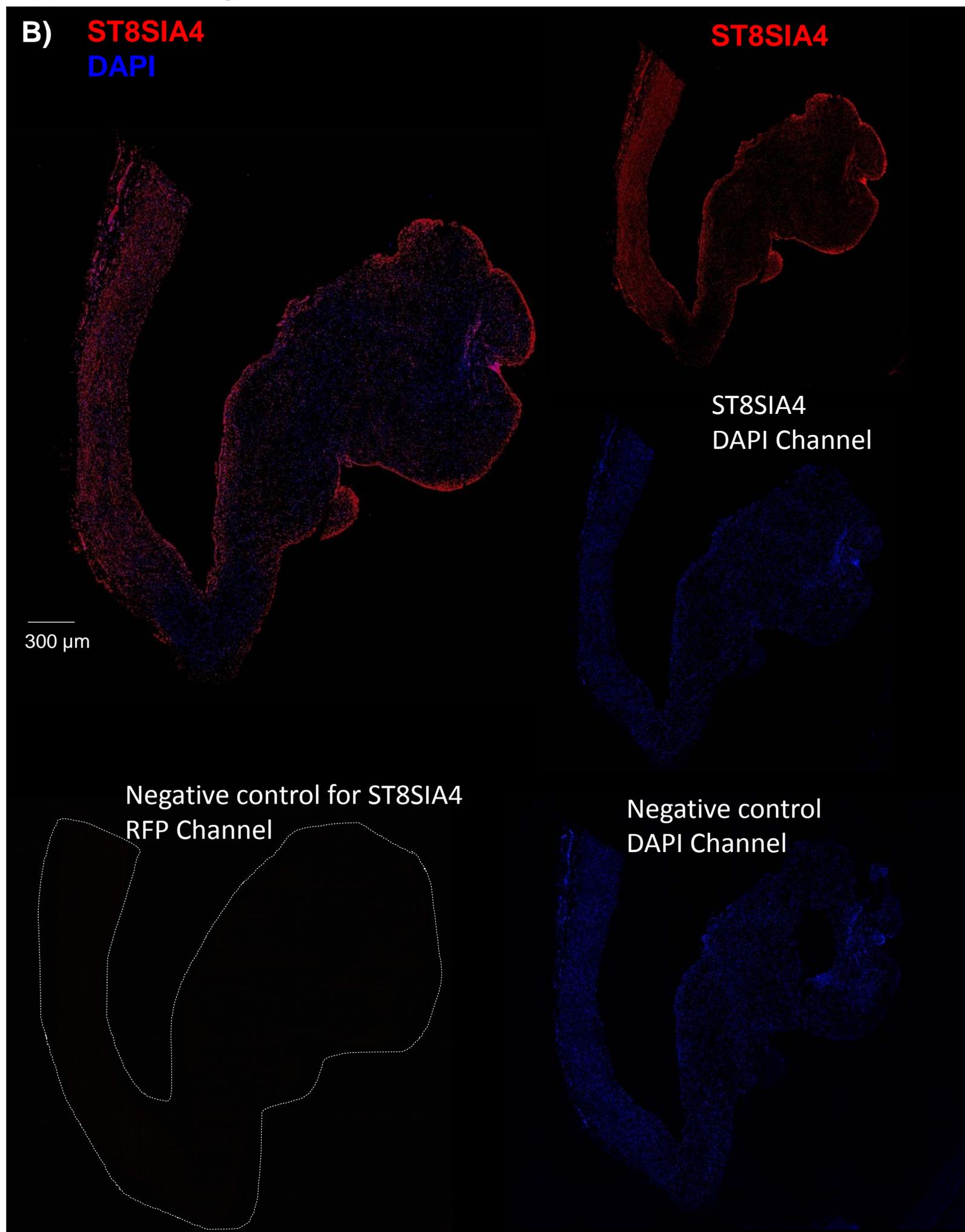
Supplemental Figure 5. Evaluation for age-related changes in AS valves due to sialic acid containing N-glycans. A) Normalized peak data were extracted per valve and compared by age ≤ 5 and >5 years of age per pediatric guidelines. By Mann Whitney U p value, there was no significant difference by age within the AS group. B) Comparison of all pixel data per valve. Pixel data is peak intensity normalized to total ion current for all valves in the dataset. Older valves show more variability that includes higher intensity pixels. However, the cumulative average taken across the entire tissue show similar levels of expression.

Supplemental Figure 6A.

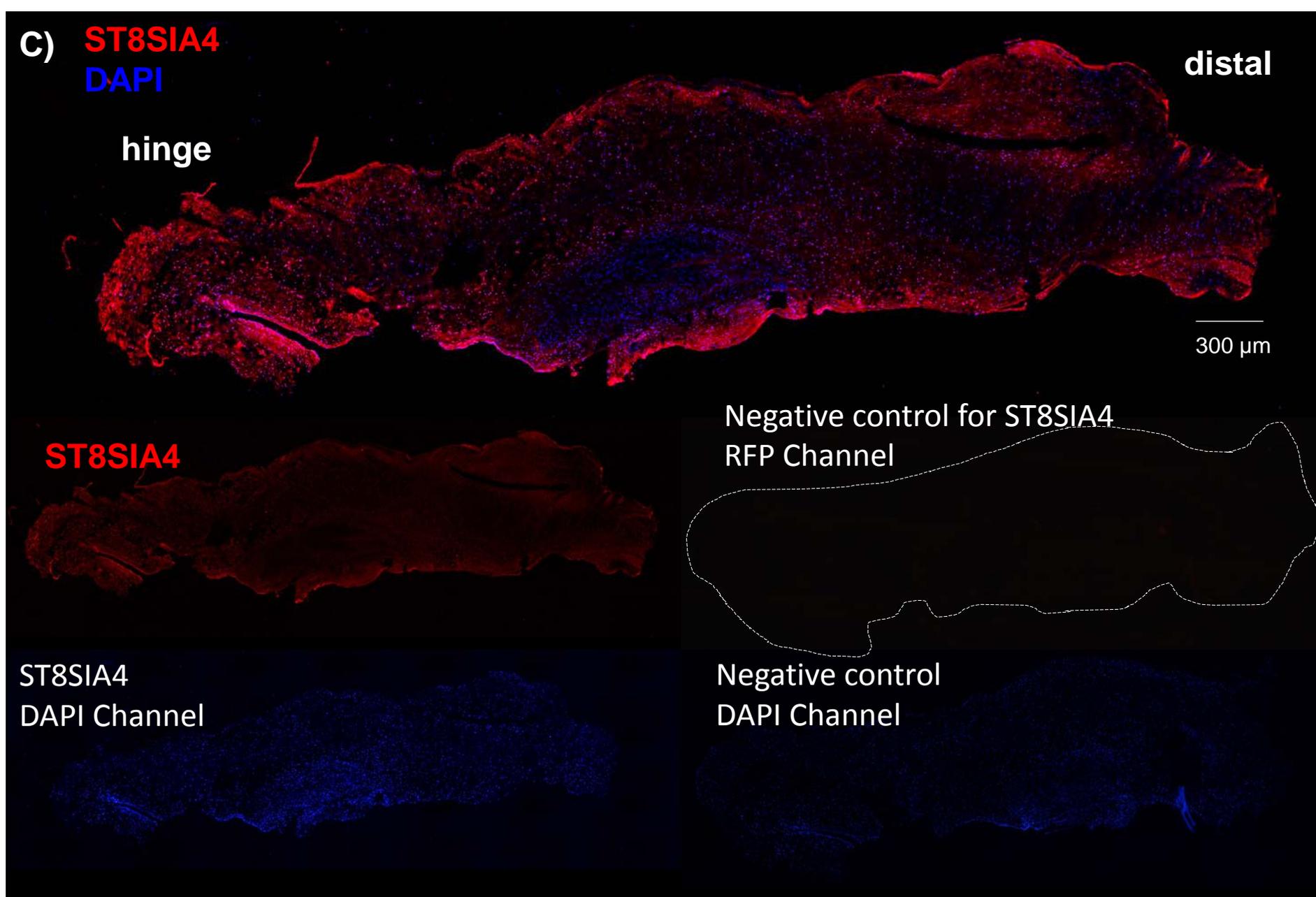


Supplemental Figure 6A. A) 2 year old aortic valve with normal function stained for ST8SIA4, a sialyltransferase that adds consecutive α 2,8 sialic acids.

Supplemental Figure 6B.

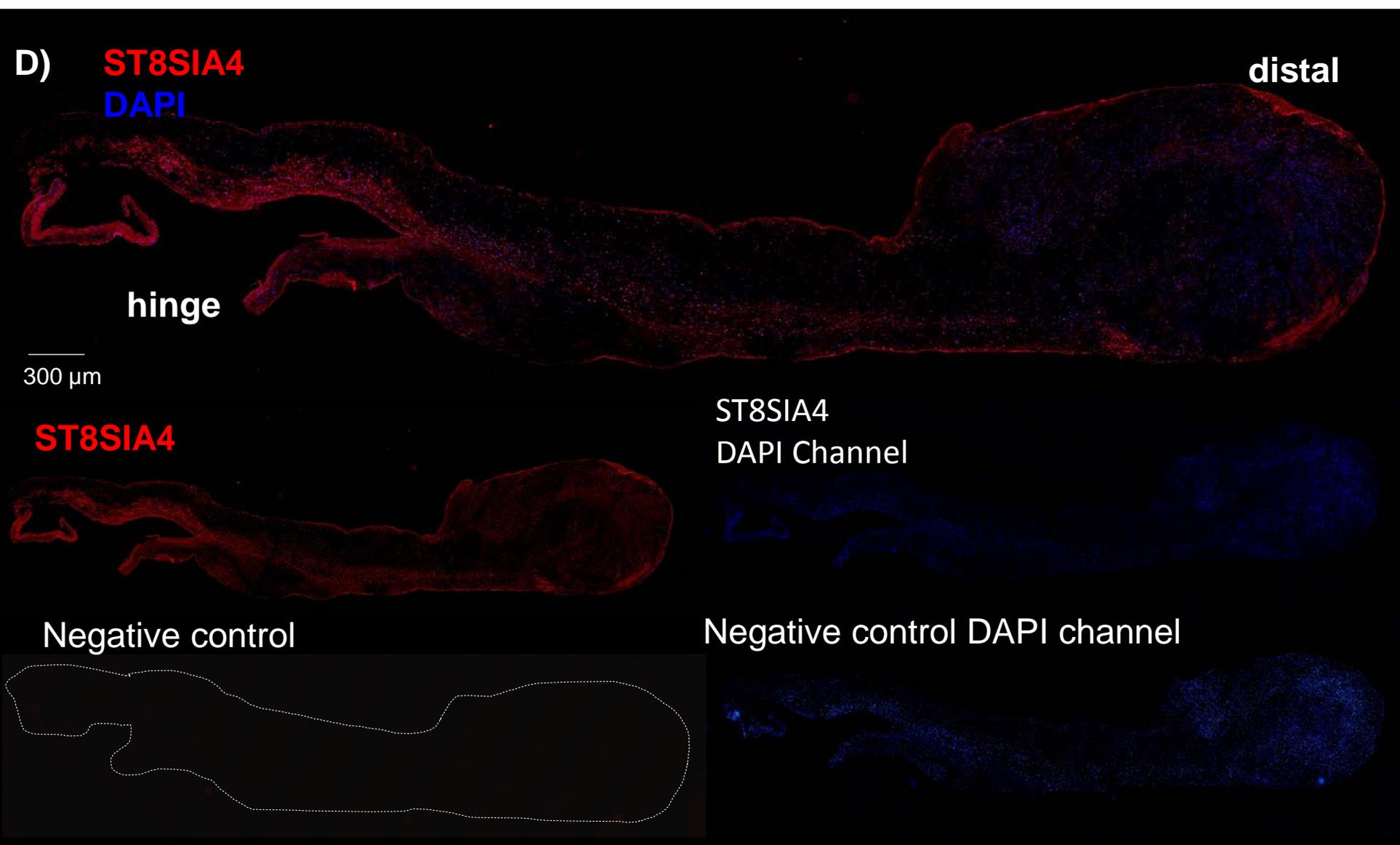


Supplemental Figure 6B. 0.04 year old bicuspid aortic valve with stenosis and AVI stained for ST8SIA4, a sialyltransferase that adds consecutive α 2,8 sialic acids. The negative control was performed at the same time on a neighboring section of the same valve.



Supplemental Figure 6C. 5 year old bicuspid aortic valve with stenosis and AVI stained for ST8SIA4, a sialyltransferase that adds consecutive α 2,8 sialic acids. The negative control was performed at the same time on a neighboring section of the same valve.

Supplemental Figure 6D.



Supplemental Figure 6D. 17 year old bicuspid aortic valve with stenosis and AVI stained for ST8SIA4, a sialyltransferase that adds consecutive α 2,8 sialic acids. The negative control was performed at the same time on a neighboring section of the same valve.

